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THE STREPTOCOCCI FROM SCARLATINAL AND NORMAL THROATS AND FROM OTHER SOURCES.*

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INTRODUCTION.

IT is a well-established fact that streptococci are quite constantly found in great abundance on the tonsils of scarlet-fever patients. This is shown very clearly by the investigations of Weaver, Baginsky and Sommerfeld, Charlton, Charbade, Booker, Tangl, Klein, Kurth, and others. It is also well known that these organisms are frequently found on the tonsils of healthy persons, although not in such great abundance as on the tonsils of scarlet-fever patients. Hilbert, Tunnicliff, Dungern, Schweighofer and Widal, and Bezancon state that they found them in all normal throats which they examined. Others, however, did not find them so constantly. Black found them on only 30 per cent of the normal tonsils which he examined. Netter found them in 5.5 per cent; Kurth in 8 per cent; Podbielsky in 2 per cent; and Dornberger in 45 per cent of the normal throats examined by them. In most of these investigations no detailed study was made of the streptococci that were found. In some investigations glucose-broth tubes were inoculated with material from the tonsils, and if cocci were found in chains after incubation of the tubes, it was concluded that streptococci were present. Apparently no further study was made of the cultures. In consideration of this fact it was thought worth while to take up this question again and make a more detailed study of the different strains of streptococci and diplococci which can be isolated from scarlatinal and normal throats.

METHOD OF STUDY.

Through the researches of Schottmüller[1] and of Rosenow[2] we know that pneumococci produce green colonies in blood-agar plates, while *Streptococcus pyogenes* produces small grayish colonies which

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* Received for publication August 2, 1906.

[1] *Münch. med. Wchnsch.*, 1903, 50, p. 909.

[2] *Jour. Infect. Dis.*, 1904, 1, p. 308.

are surrounded by a pefectly clear area of hemolysis. Schottmüller also pointed out the fact that there are streptococci which produce green colonies in blood-agar plates, very similar to the pneumococcus colonies. In this study I have made use of these facts in isolating *Strept. pyogenes* from material obtained from the tonsils. A sterile cotton swab was rubbed against the tonsils in such a manner as to avoid the tongue so much as possible. The swab was then rinsed in 1–1.5 c.c. of sterile broth, and the latter was used immediately to inoculate three or four blood-agar tubes and the same number of litmus inulin-agar tubes which were poured into plates and incubated for 24 to 48 hours at 36° C. Blood-agar was prepared by melting plain agar tubes, cooling to 45° C., and adding 0.5 c.c. of sterile defibrinated rabbit or human blood to each tube. Litmus inulin-agar was prepared as previously described.[1]

After 24 hours' incubation it is possible to pick out the streptococcus colonies in the blood-agar plates as they are small gray colonies surrounded by a perfectly clear area of hemolysis. It must be remembered, however, that diphtheria bacilli and pseudodiphtheria bacilli may produce hemolyzing colonies which closely resemble the streptococcus colonies. Furthermore, I have occasionally found pneumococcus colonies which produce considerable hemolysis and may therefore be mistaken for *Strept. pyogenes* colonies. The pneumococcus colonies are not easily recognized in these plates as there are always found in the throat many chain-forming cocci which form green colonies like the pneumococcus colonies. In all instances subcultures were made from four to eight hemolyzing colonies and the same number of green colonies. These cultures were studied morphologically and culturally and their fermentative power was tested in Hiss's[2] inulin serum-water medium, or in a slightly modified medium which was prepared as follows:

Dissolve 5 gr. of NaCl, 20 gr. of peptone (Witte) and 20 gr. of pure inulin in 1,000 c.c. of distilled water. Add 20 c.c. of a 5 per cent solution of pure litmus and tube, putting 2 c.c. of the mixture into each tube, and sterilize in the autoclav. After sterilization, add (with a sterile pipette) 2 c.c. of sterile heated ascites fluid,* or preferably heated beef serum, to each tube and incubate for 24 hours before using.

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 183.

[2] *Jour. Exper. Med.*, 1905, 6, p. 317.

* Great care must be observed not to use ascites fluid which contains fermentable carbohydrates. Each lot must be tested with organisms that are known to have great fermentative powers and if acid is produced it must not be used.

The beef serum was collected without taking any special precautions but was diluted with an equal volume of water and passed through a large Berkefeld filter, drawn off into 50 to 100 c.c. tubes and heated to 65° C. for one half-hour on two successive days.

This medium was preferred to Hiss's serum-water because it was found that some pneumococci and some streptococci which have recently been obtained from blood cultures do not grow well in the latter.

In the early part of this work no litmus inulin-agar plates were used, but pneumococci were looked for among the green colonies in blood-agar plates. In those cases subcultures were made and studied from 10 to 12 green colonies. Organisms were classed as typical pneumococci only: (1) When they were found to be Gram-positive cocci which grew chiefly in pairs on blood-agar slants; (2) when they fermented inulin; and (3) produced green or slightly hemolyzing colonies in blood-agar plates.

In the litmus inulin-agar plates most pneumococci produce red colonies which are easily recognized on the blue background, and as practically no other mouth bacteria produce red colonies in this medium[1] it is easy to isolate pneumococci from the mouth and throat with the aid of these plates. Subcultures were, however, always made from two or three red colonies, and the organisms positively identified. In no instance have I isolated an organism by this method which could not be classed as a pneumococcus, although some of these organisms form chains of considerable length in some liquid media, and they do not seem to have a well-formed capsule.

RESULTS.

A total of 154 throat cultures were made and examined as follows: Normal 51; scarlatinal 75; measles 14; tonsilitis 5; pneumonia 5; and laryngitis 4. The findings differ very widely and must be considered under separate heads.

Scarlatina.—All the blood-agar plates inoculated with material from scarlatinal throats contained many *Strept. pyogenes* colonies and varying numbers of green colonies. The cases may be roughly divided into 3 great groups: (1) Those from which the plates contained mostly (60 to 95 per cent) *Strept. pyogenes* colonies —31 cases. Two sets of plates contained only *Strept. pyogenes* colonies. (2) Those from which the plates contained about as many *Strept. pyogenes* colonies as green colonies—20 cases. (3) Those from which the plates contained fewer *Strept. pyogenes* colonies (15 to 40 per cent) than green colonies—21 cases. As a rule the streptococcus colonies greatly predominate over the other colonies when the inflammation of the throat is pronounced, and they rapidly decrease in number with the subsidence of the throat symptoms. When the throat symptoms are mild the proportion of *Strept. pyogenes* colonies to the other colonies is often quite small. I have met with one exception to this statement in the case of a small girl who had a very bad throat, but the plates inoculated with material from it contained far more green colonies than *Strept. pyogenes* colonies. A large proportion of the green colonies in this set of plates were typical pneumococci which proved to be highly virulent for rabbits.

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[1] *Jour. Infect. Dis.* 1906, 3, p. 183.

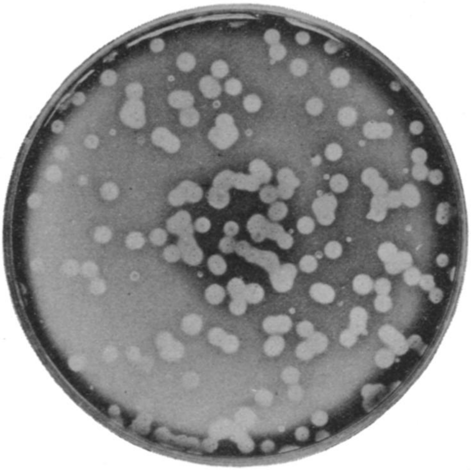

FIG. 1. — Photograph of a blood-agar plate inoculated with material from a scarlet-fever throat. Many *Strept. pyogenes* colonies and few small green colonies.

Pneumococci were not looked for in four cases but were found in 64 of the 71 throats that were examined for them—91.4 per cent. Diphtheria bacilli were found four times and *B. mucosus* and *B. influenzae* once. *Staph. aureus* and *citreus* and *M. tetragenus* were found occasionally, but these were probably contaminations from the tongue. Gram-negative diplococci which form small brownish colonies were found quite frequently. These were not always studied in detail, but some were identified as *M. catarrhalis*.

Normal throats.—All blood-agar plates inoculated with material from normal throats contained many green and slightly hemolyzing greenish colonies. *Strept. pyogenes* colonies were found in 30 of 51 throats which were studied, but were never present in large numbers (see Fig. 2) and were entirely absent in 21 cases (41.2 per cent). In only five sets of plates did I find more than 10 per cent of *Strept. pyogenes* colonies, and in 15 sets there were only 1 per cent or less of these colonies. Not infrequently one finds hemolyzing colonies in blood-agar plates which, upon closer study, are found to be organisms that produce greenish colonies in subsequent plates. Great care must therefore be exercised in deciding on the presence or absence of *Strept. pyogenes* colonies. The slightly hemolyzing, often greenish colonies also are very puzzling and will be described more fully on a later page. Pneumococci were found 42 times (82 per cent), and diphtheria bacilli three times. Gram negative diplococci and staphylococci were found quite frequently and *B. mucosus* once.

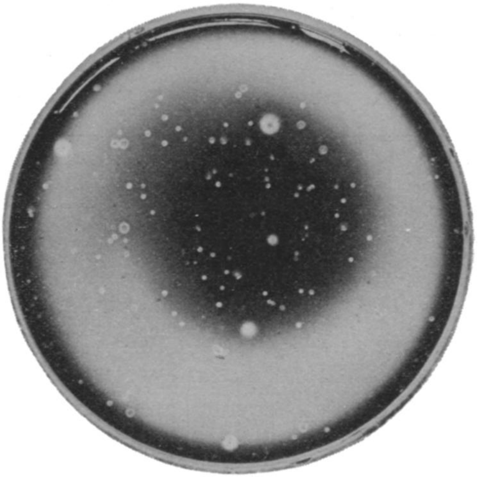

FIG. 2.—Photograph of a blood-agar plate inoculated with material from a normal throat. Many small greenish colonies and four *Strept. pyogenes* colonies.

Measles throats.—The findings in the throats of measles patients corresponded closely to those of normal throats. *Strept. pyogenes* was found in 9 of 14 throats which were examined (65 per cent) and pneumococci were found in 12 (85.7 per cent).

Tonsilitis.—*Strept. pyogenes* colonies always predominated in these plates, but green colonies also were present. Pneumococci were found in four of the five cases examined, but were not specially looked for in the other case. In one case influenza bacilli were found in great abundance.

Pharyngitis.—Four cases of so-called pharyngitis were examined, but none of the blood-agar plates inoculated with material from these throats contained *Strept. pyogenes* colonies. All plates contained many green colonies and some contained small brownish colonies.

The latter were chiefly Gram negative cocci, but in one case they were influenza bacilli. Pneumococci were isolated from all of these cases. The finding of pneumococci in such a large per cent of throats of persons not afflicted with pneumonia is in agreement with the work of the Commission for the Investigation of Acute Respiratory Diseases of the Health Department of the City of New York.[1]

Pneumonia.—Three of the five sets of plates inoculated with material from the tonsils of pneumonia patients contained very few *Strept. pyogenes* colonies, and the other two sets did not contain any. All blood-agar plates contained many green colonies and the litmus inulin-agar plates contained many red colonies. Typical pneumococci were easily isolated from all plates.

It will be noticed that green colonies were found in all blood-agar plates except two sets which were inoculated with material from scarlatinal throats. Only a small proportion of these colonies are pneumococci and the remainder form a large group of organisms between the typical pneumococci and streptococci. Some of these organisms seem to correspond to Schottmüller's *Strept. viridans*, but others are very closely related to the pneumococcus. In addition to the green colonies there are often found slightly hemolyzing colonies which may sometimes have a green tint. These colonies, which are more abundant in plates inoculated with material from normal throats and from throats of measles patients than in plates inoculated with material from scarlatinal or tonsilitis throats, must not be confounded with the *Strept. pyogenes* colonies. Rarely one finds chain cocci in throats which form hemolyzing colonies in plates of plain agar and blood, and green colonies of the pneumococcus type in glucose blood-agar plates. These organisms I have found in the throats of two measles patients and once in the throat of a healthy man. All these organisms, including the *Strept. viridans*, are closely related to one another and form a great group which can be sharply differentiated from the *Strept. pyogenes* by the following characteristics:

1. The cocci found in smears made from milk cultures stain rather poorly and unevenly with Lœffler's methylene blue, are usually somewhat elongated or lance-shaped and often are found in pairs

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[1] *Jour. Exper. Med.*, 1905, 7, p. 401.

in the chains. On the other hand, *Strept. pyogenes*, when grown in litmus milk, takes the methylene blue stain very readily, the cocci are never distinctly grouped in pairs in the chains, and are not elongated, but frequently appear to be disc-shaped. On account of this peculiarity the cocci in the chains of *Strept. pyogenes* appear closely packed together, while those of *Strept. viridans* and allied organisms are relatively far apart and the chains have the appearance of having been stretched. This grouping in pairs in the chains is shown in Fig. 3.

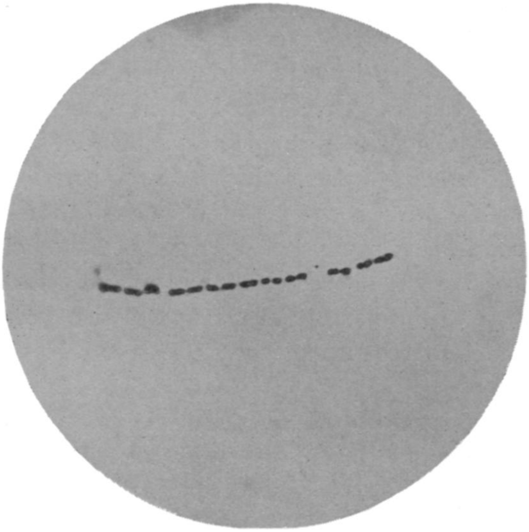

FIG. 3.—*Strept. viridans.* Cocci are elongated and arranged in pairs in the chain. Microphotograph × 1,500.

2. On ascites-agar slants a large proportion of these atypical organisms form lance-shaped or oval cocci which are grouped chiefly in pairs, but also in short chains. A grouping in pairs within the chains is usually observed. Some of the cocci have well-formed

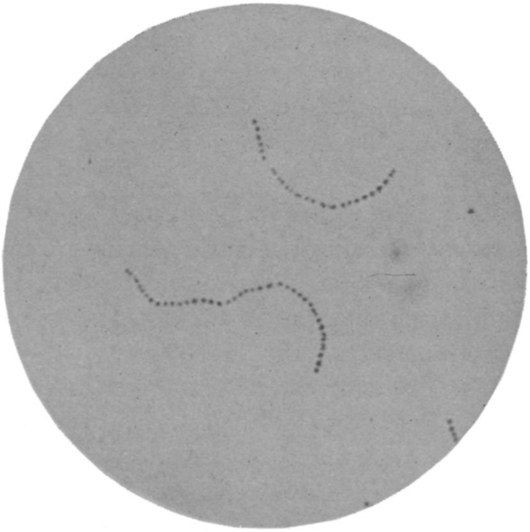

FIG. 4.—*Strept. pyogenes.* Cocci are not elongated and are not arranged in pairs in the chains. Microphotograph × 1,500.

capsules and might be classed as pneumococci, although they do not ferment inulin and usually form chains in liquid media.

3. When pure cultures of these organisms are grown on serum-glucose-agar slants (three parts of glucose agar and one part of ascites fluid or serum) no change is produced in the medium, while *Strept. pyogenes* turns it white and opaque in 36 to 48 hours. This interesting reaction was first observed by Libman[1] who used it to differentiate the pneumococcus from *Strept. pyogenes*. Libman's observation is correct as far as it goes, but it does not go far enough, because there is a large group of cocci which cannot be classed as pneumococci although their behavior on the serum-glucose-agar is like that of typical pneumococci. It must be stated, however, that most of these organisms are more closely related to the pneumococcus than to *Strept. pyogenes*.

Something over 1,500 milk cultures of different strains of chain-forming cocci from throats and other sources were examined microscopically and classed either as *Strept. pyogenes* or as atypical forms. Seventy-five strains which had thus been identified as typical *Strept. pyogenes*, 77 strains which had been classed as atypical forms, and 16 strains of pneumococcus were planted on serum-glucose-agar slants and incubated for three days. The results are shown in Table 1. Seventy-four of the 75 strains of *Strept. pyogenes* turned the medium white and opaque in 48 hours, while only four of the atypical forms and one pneumococcus produced a similar change in three days. Three of the four atypical forms which changed the culture medium were isolated from the same throat (normal). These produce slight hemolysis in blood-agar plates and readily ferment inulin, which indicates that they are not typical *Strept. pyogenes*. The fourth strain forms green colonies in blood-agar plates, the cocci are round, often arranged in pairs, in chains of 8 to 16 cocci, and have a well-formed capsule.

The fact that some of the atypical forms produce green colonies while others form hemolyzing colonies in blood-agar plates may suggest to some that they are very different organisms. This does not necessarily follow, because I have shown that the green coloration is dependent on the production of acid and the action of this

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[1] *Jour. Med. Res.*, 1901, 1, p. 84.

TABLE 1.

THE EFFECT OF *Streptococcus pyogenes*, PNEUMOCOCCI, AND CULTURES OF INTERMEDIATE COCCI ON SERUM-GLUCOSE-AGAR.

Streptococcus pyogenes.

Source	No. of Races Tested	Character of Colonies in Blood-Agar Plates	Effect on Serum-Glucose-Agar
Scarlet-fever throats	29	Hemolyzing	White opacity
Measles throats	4	"	" "
" "	1	"	No change
Normal throats	18	"	White opacity
Tonsilitis	2	"	" "
Otitis media	4	"	" "
Erysipelas	4	"	" "
Hand infection	4	"	" "
Puerperal sepsis	2	"	" "
Meningitis	1	"	" "
Conjunctivitis	1	"	" "
Phlegmon of the leg	1	"	" "
Suppurative lymphadenitis	2	"	" "
Frontal sinus infection	1	"	" "
Pericardial fluid (body dead of scarlatina)	1	Hemolyzing	White opacity

ATYPICAL FORMS.

Source	No. of Races Tested	Character of Colonies in Blood-Agar Plates	Effect on Serum-Glucose-Agar
Scarlet-fever throats	8	Green	No change
" " "	2	Hemolyzing; greenish	" "
" " "	1	Green	White opacity
Measles throats	1	"	No change
" "	1	Green in glucose-blood-agar; hemolyzing in plain-blood-agar plates	" "
Tonsilitis	3	Green	No change
Normal throats	18	"	" "
" "	30	Slightly hemolizing; not green	" "
Normal throats	7	Hemolyzing; greenish	No change
" "	1	Green in glucose-blood-agar: hemolyzing in plain-blood-agar plates	" "
Normal throats	3	Slightly hemolyzing	White opacity
Otitis media (mixed infection)	2	Green	No change

PNEUMOCOCCI.

Source	No. of Races Tested	Character of Colonies in Blood-Agar Plates	Effect on Serum-Glucose-Agar
Normal throats	4	Hemolyzing greenish	No change
" "	4	Green	" "
" "	1	"	White opacity
Scarlet-fever throats	2	"	No change
Conjunctivitis	1	"	" "
Blood cultures from pneumonia patients	4	"	" "

acid on the red corpuscles.[1] When the culture secretes hemolysin rapidly the red corpuscles about the colony are destroyed early and no green color is seen even if a considerable amount of acid is produced. On the other hand, some cultures of *Strept. pyogenes* which produce hemolyzing colonies in plain blood-agar plates form green colonies in plates of glucose-agar and blood. Furthermore, the secretion of hemolysin by bacteria is a variable character. It is very pronounced in some strains of streptococcus, rather feeble in others, and may be absent entirely in others. I have two strains of streptococcus which have lost this property entirely by being grown in glucose broth for about two years. They now produce brownish colonies in plain blood-agar plates and greenish colonies in plates of glucose-agar and blood.

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 663.

THE FERMENTATIVE POWER OF STREPTOCOCCUS PYOGENES

The fermentative power of 124 races of *Strept. pyogenes* from various sources was tested on the following carbohydrates: dextrin, inulin, lactose, maltose, raffinose, rhamnose, saccharose, and salicin; and on the polyatomic alcohols mannit and dulcit. The culture medium used was that described on p. 756 except that the carbohydrate and peptone solutions were sterilized by the fractional method* before the addition of the serum. Sterile serum which had been heated to 65°–70° C. was added to each tube after sterilization. In this medium all strains fermented dextrin, lactose, maltose, saccharose, and salicin, but none fermented inulin, raffinose, rhamnose, and dulcit. Occasionally a strain would not readily ferment dextrin or saccharose, but on repeated trials it would ferment them. These substances, therefore, did not reveal any differences between the various races of streptococcus used.

The hexatomic alcohol, mannit, however, was found to be fermented quite readily by some races and not at all by others. This substance was used then to divide the various races of *Strept. pyogenes* into two classes, (1) those which ferment mannit and (2) those which do not ferment it. The chief points of interest are shown in Table 2. Nearly all these cultures were obtained from a single colony in a blood-agar plate which had been inoculated with material from an infected region. In many instances subcultures from two or three colonies in the same plate were tested, and it was found that these results always agreed. In no instance did I get two strains from the same infection which did not have the same effect on mannit. Five strains were tested 3 times, at intervals of 2 to 3 months, and the results were always the same. The table therefore represents less than one-half of the tests that have actually been made.

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* It is sometimes almost impossible to sterilize some of these carbohydrates by the fractional method. After a great many failures I hit upon the scheme of sterilizing twice on the third day. This makes four sterilizations with only a short space of time between the last two. By this method I hardly ever failed to get every tube sterile.

TABLE 2.

CLASSIFICATION OF STREPTOCOCCI ACCORDING TO THEIR EFFECT ON MANNIT.

Source of Streptococci	No. of Races Tested	Mannit Fermented	Mannit not Fermented
Scarlatinal throats	60	37	23
Normal throats	19	6	13
Measles throats	6	1	5
Tonsilitis throats	6	3	3
Phlegmon of the leg	3	1	2
Erysipelas	7	4	3
Otitis media (post-scarlatinal)	6	4	2
Otitis media (measles)	1	..	1
Hand infection	4	..	4
Pericardial fld. (body dead of scarlatina)	2	2	..
Suppurative lymph-adenitis (complicating scarlatina)	1	..	1
Suppurative lymph-adenitis (complicating erysipelas)	2	1	1
Mastoid abscess	1	..	1
Frontal sinus infection	1	1	..
Meningitis	1	..	1
Conjunctivitis (scarlatinal)	1	1	..
Cervix uteri (puerperal sepsis)	1	1	..
Blood culture (puerperal sepsis)	1	1	..
Arm infection	1	..	1
Totals	124	63	61

To obtain uniform results it is necessary that the culture medium should always be prepared in the same manner. If the nutritive qualities of the culture medium are allowed to vary one can hardly expect to get uniform results. A number of preliminary tests with Hiss's serum-water medium which had been sterilized at 100° C. showed a considerable lack of uniformity. This was thought to be due to the fact that some of the cultures grew rather poorly in this medium.

Although these tests show a difference in the fermentative power of different races of *Strept. pyogenes*, it will be noticed that there is no agreement between the source of the culture and its effect on mannit. Further differences between the organisms of these two classes were now looked for by means of agglutination tests with immune serum.

AGGLUTINATION.

Three sheep were immunized with different strains of streptococci as follows: Sheep "A" with a mannit-fermenting and Sheep "B" with a non-fermenting *Strept. pyogenes;* Sheep "C" with a *Strept. viridans.* The sheep were injected every 10 days, for a period of four months, first with heated cultures and later with living cultures, until 30 c.c. of a 48 hour calcium broth culture was borne without bad effects. Agglutination tests with the sera of these sheep showed that not all mannit-fermenting streptococci are agglutinated by the serum of the sheep which had been immunized with a mannit-fermenting organism, while some of the non-fermenters are agglutinated by that serum. Similarly, not all non-fermenters are agglutinated by the serum of the sheep which was immunized with the non-fermenting organism. None of the strains of the *Strept. pyogenes* are agglutinated by the serum of the sheep immunized with the *Strept. viridans.*

All tests were macroscopic, using 72 hour calcium broth cultures according to the method described by Hiss.[1] The tubes were incubated for three hours and then set into the ice chest for 12 hours before recording the results. The details are shown in Table 3.

The tests show, moreover, that many strains of *Strept. pyogenes* coming from scarlet fever are agglutinated by the serum of that sheep (Sheep "A") which had been immunized with a streptococcus isolated from a scarlet-fever throat. These organisms are not agglutinated by the serum of Sheep "B" which was immunized with a streptococcus isolated from a phlegmon of the leg. Of the 11 strains which were obtained from scarlet-fever patients, three (381, Laura, and Morris) were not agglutinated by the serum of Sheep "A" in higher dilutions than by the serum of Sheep "B."

TABLE 3.
AGGLUTINATION OF STREPTOCOCCI BY
1. SERUM OF SHEEP "A."

STREPTOCOCCI	DILUTIONS				
	1:50	1:100	1:200	1:300	1:500
Mannit Fermented—					
Frances	+++	+++	+++	+++	+++
Anna	+++	+++	+++	++	++
Duffy	+++	+++	+++	+++	++
381	+	o	o		
Smyth	o	o	o		
Goldspohn	+++	++	+		
Stuart	+++	++	+		
Mannit not Fermented—					
Coburn	+	o	o		
Morris	o	o	o		
Geragass	++	o	o		
Joe R.	+++	+++	++	++	+
Henry R.	+++	++	+	o	
Laura	o	o	o		
Harris	++	+	+	o	
Minte	+	o			
C. T. N.	++	++	o		
G 2	o	o	o		

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[1] *Jour. Exp. Med.*, 1905, 7, p. 560.

2. SERUM OF SHEEP "B."

Mannit Fermented—					
Frances	o				
Anna	o				
Duffy	o				
381	+				
Smyth	o				
Goldspohn	o				
Stuart	++	++	+	o	
Mannit not Fermented—					
Coburn	+++	+++	+++	+++	+++
Morris	o				
Geragass	+	o			
Joe R.	o				
Henry R.	o				
Laura	o				
Harris	o				
Minte	o				
C. T. N.	o				
G 2	o				

3. SERUM OF SHEEP "C."

Mannit Fermented—					
Frances	o				
Anna	o				
Duffy	o				
381	+				
Smyth	o				
Goldspohn	o				
Stuart	++	+			
Mannit not Fermented—					
Coburn	o				
Morris	o				
Geragass	o				
Joe R.	o				
Henry R.	o				
Laura	o				
Harris	o				
Minte	o				
C. T. N.	o				
G 2	+++	++	+		

Sheep "A" immunized with Streptococcus Frances.
Sheep "B" immunized with Streptococcus Coburn.
Sheep "C" immunized with *Strept. viridans* (G 2).

Source Streptococci "Frances," "Joe R.," "Henry R.," "Harris," from scarlet-fever throats; "Duffy" and "381" from pericardial fluid of scarlet-fever bodies; "Anna," "Goldspohn," and "Laura" from scarlatinal otitis media; "Smyth" from uterus in puerperal sepsis; "Stuart" from erysipelas; "Coburn" from phlegmon of the leg; "Morris" from suppurating lymph gland in scarlatina; "Geragass" from abscess in erysipelas; "C. T. N." from tonsil in tonsilitis; "G 2" is a *Strept. viridans* from a scarlet-fever throat.

Organisms "381" and "Laura" had each passed through more than 15 rabbits. Organism "Morris" was obtained from suppurating cervical glands of a very mild case of scarlatina. The cervical adenitis did not appear until the end of the fourth week, after the patient had been allowed to be out of bed for more than two weeks.

The serum of Sheep "B" agglutinated only the homologous organism.

It is not necessary here to go into the literature on agglutination of streptococci, but mention should be made of the work of Rossiwall and Schick[1] who found that Moser's antistreptococcus serum agglutinated many strains of streptococcus coming from scarlet-fever patients. They call special attention to an organism obtained from a case of surgical scarlatina which was agglutinated by Moser's serum in high dilutions. From the throats of some scarlatinal patients they isolated streptococci which were agglutinated by that serum and some that were not agglutinated by it. It must be recalled that Moser's serum is prepared by immunizing horses with streptococci that are isolated from scarlet-fever patients, without passing the organisms through animals.

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[1] *Wiener klin. Wchnschr.*, 1905.

VIRULENCE OF STREPTOCOCCI AND PNEUMOCOCCI ISOLATED FROM THROATS.

Most cultures of *Strept. pyogenes* and some pneumococci isolated from scarlatinal and normal throats have some virulence for rabbits, but *Strept. viridans* has practically no virulence for these animals. Thirty-one rabbits weighing from 900 to 1,200 G. were injected ntraperitoneally with *Strept. pyogenes* cultures as follows: Four rabbits with a suspension of two 48 hour blood-agar slant cultures of organisms isolated from normal throats. Three of these animals died and one remained well. Twelve rabbits, with the same kind of a suspension of organisms isolated from scarlatinal throats. Eight of these animals died and four remained well. Eight rabbits, with a suspension of one blood-agar slant culture of organisms isolated from normal throats. Five of these animals died and three remained well. Seven rabbits, with a suspension of one blood-agar slant culture of organisms isolated from scarlatinal throats. One of these animals died and six remained well.

These results indicate that streptococci isolated from normal throats are slightly more virulent for animals than those isolated from scarlatinal throats. This is in perfect agreement with the results obtained by Hilbert[1] and Tunnicliff.[2]

Strept. viridans has a lower virulence for rabbits than *Strept. pyogenes*. Nine rabbits weighing from 900 to 1,200 G. were injected into the peritoneum with two blood-agar slant cultures of different strains of these organisms, but only one of these animals died.

Ten rabbits were injected with two blood-agar slant cultures of different strains of pneumococci isolated from normal and scarlatinal throats. Four of these animals died and the other six remained well. Fourteen rabbits were then injected with larger doses, but only five of these died. There are no indications that pneumococci in scarlatinal throats are more virulent than those in normal throats, with the one exception referred to above.

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[1] *Zeitschr. f. Hyg.*, 1899, 31 p. 381.

[2] *Trans. Chicago Path. Soc.*, 1904, 6, p. 175.

CONCLUSIONS.

Strept. pyogenes is constantly found in great abundance on the tonsils of patients suffering from tonsilitis and scarlet fever, before the inflammation of the throat has subsided. These organisms rapidly decrease in numbers with the subsidence of the throat symptoms.

Strept. pyogenes cannot be considered a normal inhabitant of all healthy throats, although it was found in small numbers in 59 per cent of the normal throats in this series.

Pneumococci of low virulence were found in 119 of this series of 154 throats.

A large group of organisms which lies between the typical *Strept. pyogenes* and the pneumococcus was found in all normal throats and in nearly all diseased throats. These organisms have very little virulence for rabbits, and, as they are found in great abundance in practically all throats, they appear to be normal inhabitants of the throat. These organisms can be sharply differentiated from *Strept. pyogenes* both morphologically and by their effect on serum-glucose-agar.

Cultures of *Strept. pyogenes* can be divided into two classes according to their fermentative power: (1) those which ferment mannit, and (2) those which do not ferment it. Not all inulin-fermenters are agglutinated by the serum of a sheep immunized with an inulin-fermenting streptococcus, while some non-fermenters are agglutinated by it. Most streptococci coming from scarlet-fever patients are agglutinated in fairly high dilutions by the serum of a sheep immunized with an organism of that source.

Strept. pyogenes from normal throats appears to have a slightly greater virulence for rabbits than when obtained from scarlatinal throats.

I am indebted to Professor Hektoen for many suggestions and to Dr. W. L. Baum for the privilege of getting throat cultures from patients under his care at the Cook County Hospital.